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Effects of pH on Biomass Allocation in Acer rubrum Seedlings

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Effects of pH on Biomass

Allocation in *Acer rubrum* Seedlings

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By

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APPROVAL PAGE

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CHAPTER I

INTRODUCTION

The dominance of certain tree species in an area is the result of myriad influencing factors. Individual genetic make-up has evolved over time to maximize each particular species' success in a given area. The large area covered by the deciduous forests of the eastern United States presents a truly unique ecosystem. There are basic environmental ranges consistent throughout this area that contribute to the success of certain deciduous associations. Precipitation ranges from 75 centimeters in the Great Lakes region to 150 centimeters along the Gulf Coast, and 125 centimeters along the Atlantic Coast to 85 centimeters at the grassland-forest boundary (Vankat, 1979). Temperature disparities across the region range such that snowfall is common in the north but rare in the south, and the growing season may be 120 days to the north but 250 days to the south (Vankat, 1979). Two soil types divide this forest - to the north are found Grey-Brown Podzolic soils and to the south are Red-Yellow Podzolic soils (Vankat, 1979).

The primary constituent of all plants of the deciduous forest is water. The pH of local water supplies has a great impact on the proper functioning of chemical reactions essential to life within the trees' tissues. There is a great deal of susceptibility to alterations in the pH of this supply. Understanding what constitutes acid rain is key to forming an introductory comprehension of the concepts presented in the current study. In pure water, hydrogen and hydroxide ions combine to form H_2O, which has no charge (polar in nature) (Bush, 2000). It is the abundance of non-bonded hydrogen ions that determines the pH of precipitation. In neutral solution, approximately 1 in 10 million H^+ ions are non-bonded (Bush, 2000). The scale for pH is logarithmic, hence, one unit of change on the pH scale equates to a 10-fold change in the number of non-bonded H^+ ions (Bush, 2000). Neutral on this scale is 7; higher measures are increasingly basic, and lower measures are increasingly acidic. Normal precipitation is slightly acidic, having a pH of approximately 5.6 due to combination with CO_2 in the atmosphere, yielding carbonic acid (Bush, 2000). The primary sources of pollutants in the atmosphere contributing to acid rain are sulfur dioxide (SO_2) from coal burning power plants, nitric oxide (NO), and nitrogen dioxide (NO_2), resulting from combustion of gasoline (Bush, 2000). These combine in the atmosphere with H_2O molecules to form H_2SO_4 (sulfuric acid) and HNO_3 (nitric acid).

The implications of acidified precipitation, for regional vegetation, may range from yellowing of the foliage to death. The ability of local soils to neutralize the acid is key to determining what the ramifications may be. Soils generally have a negative charge resulting from clay minerals that attract and hold positive ions (Bush, 2000). Calcium, an alkali, will buffer the impact of increased acidity, if it is present in soils. The cation exchange capacity (CEC) of a particular soil refers to the ions currently held by a soil being replaced by the H^+ ion. Ions are held in soil and displaced in the following order: aluminum (Al^{3+}) > hydrogen (H^+) > calcium (Ca^{2+}) > magnesium (Mg^{2+}) > potassium (K^+) > ammonium (NH^{4+}) > sodium (Na^+). Approximately 70% of the soils of the eastern United States are low in calcium content; for example, the siliciclastic (conglomerates, sandstones, phyllites, and quartzites) bedrock of Shenandoah National Park in Virginia has been used up with little potential remaining to neutralize more acid precipitation (Badger, 1999). When saturated with H^+ ions, aluminum, normally bound up in aluminum dioxide (Al_2SO_3), is released and becomes reactive in the soils (Bush, 2000). Aluminum is highly toxic to a large variety of plants. The trees themselves can speed this process along as they will pump out any excess H^+ ions while taking in Ca^{++} ions for neutralization, increasing the soil H^+ ions and accelerating the CEC release of aluminum (Bush, 2000).

STATEMENT OF THE PROBLEM

The problem of this study was to determine the short-term impacts of lowered pH precipitation on above and belowground biomass allocation in *Acer rubrum* seedlings.

RESEARCH HYPOTHESIS

Through the analysis of the data collected from the uniform growing parameters across all control and treatment pottings, the following hypothesis was considered:

H_1: From a neutral pH of 7, there is a positive correlation between the lowered pH of precipitation and the above and belowground biomass allocation in *Acer rubrum* seedlings.

BACKGROUND AND SIGNIFICANCE

First coined in 1850 by Robert Angus Smith, the term 'acid rain' evolved from air chemistry investigations in Britain's industrial belt (Bush, 2000). Smith discovered that the emissions from industry polluted the air with soot, impacting the chemistry of precipitation, causing it to become acidic. To this day there is still a great deal of debate over the cumulative impact anthropogenically caused acid rain has had on our environment.

Research on the pH of precipitation across the eastern United States from 1955–1996 has revealed changes in pH over time. In 1955 a range of 5.6-4.52 was noted in rather uniform concentric bands (Bush, 2000). The data for 1985 reveal the vast majority of the east in the pH range of 4.6-4.2 (Bush, 2000). Following the passage of the Clean Air Act in 1990, follow-up data in 1996 reveal significant increases in the pH with greatly reduced areas receiving rainfall below 4.3 (Bush, 2000). Currently, restrictions on coal burning power plants have been relaxed, and emissions from large areas of industry from Michigan to Ohio and Tennessee are going largely unchecked. This will undoubtedly impact future pH levels across the eastern deciduous forest range of the United States. The jet stream generally carries prevailing winds west to east. Research data from 1991-1995 reveal western parks (Yosemite, Grand Canyon, Sequoia, Kings Canyon, and Yellowstone) all received four or less kilograms of sulfate in precipitation per hectare per year (Badger, 1999). Eastern parks, on the other hand, averaged between 18 and 28 kilograms of sulfate per hectare per year (Badger, 1999).

As the pH of precipitation across the east decreases in conjunction with the poor buffering capabilities of local soils, impacts on fauna and potential ecosystem restructuring may occur. A key species across most of the eastern deciduous forest range is *Acer rubrum* (red maple). It may come to play a key role in future succession scenarios. *Acer rubrum* has the greatest north to south distribution of any east coast species and grows from swamps to dry ridges (Little, 2001). Of particular significance is the acid heartiness of the species; found locally (Norfolk, Virginia) in the pH range between 4.3-5.6, it can withstand lower readings (Day, 1987). Although many aspects of *Acer rubrum* have been examined, research on the above and belowground biomass allocation in relation to pH is lacking. The loss of *Castanea dentata* (American Chestnut) to anthropogenically introduced chestnut blight led to *Quercus* (oak) dominance. This dominance is currently receding in the face of acid rain, ozone, and pest complications (Walker, 1993). Lower pH precipitation, in combination with other changing climate variables, will likely result in hearty, fast-growing species having a dominant role in system succession (Percy, 1986). Comprehension of biomass allocation in response to acid conditions will advance the existing literature in better predicting *Acer rubrums'* potential to become a functional dominant in future systems.

LIMITATIONS

This study was limited to *Acer rubrum* seedlings provided by the Virginia Department of Forestry. All treatment and control samples were grown in identical units and experienced identical conditions of temperature, substrate, humidity, and light. Experimental greenhouse space limited the sample size per group to 20 (n=20). Due to the time parameters of this course only short-term impacts were studied, shy of a complete growing season.

ASSUMPTIONS

This research was based on the following assumptions:

1.) The seedlings provided will break dormancy.

2.) All seedlings were exposed to identical environmental conditions and were allotted identical resource availability prior to receipt.

3.) During the period of study, all samples were exposed to exactly the same conditions across the parameters of soil buffering capabilities, soil nutrient content, greenhouse humidity, greenhouse temperature, available light for photosynthesis, and amount and period of water.

4.) The only parameter of difference across all treatment and control groups was the pH of the water administered by the researcher.

PROCEDURES

Four experimental groupings of *Acer rubrum* seedlings were grown in a controlled greenhouse setting. The three treatment groups (n=20) were allotted a precipitation pH of 7, 4, and 2.5, while the control group was maintained at a pH of 5.6 (normal unpolluted rain water). Throughout the experiment data were collected on greenhouse environmental conditions of humidity, temperature, and hours of sunlight every other day. Seedlings were watered every fifth day to saturation. Soil buffering capacity was monitored every two weeks throughout the study period. Biomass was determined by measuring total dry mass for both above and belowground components, using a randomly selected sample (n=20) for initial baseline measures, and all seedlings at research completion. Prior to inducing treatment a greenhouse acclimation period of two weeks was allotted all seedlings.

DEFINITION OF TERMS

The following terms were defined to ensure consistency in this study:

Abiotic factors- "Environmental influences produced other than by living organisms, for example, temperature, humidity, pH, and other physical and chemical influences" (Bush, 2000, p. 468).

Adventitious roots- root adaptations to wet conditions, increases gas exchange and surface area supplementing nutrient and oxygen depletions (Day, 1987).

Allometry- growth of a portion of an organism relative to another portion or to the whole (Bernston & Bazzaz, 1996).

Autotroph- "An organism that obtains energy from the sun and materials from inorganic sources" (Bush, 2000, p. 468).

Biomass allocation- dry weight of living material in living organism.

Biotic factors- "Environmental influences caused by living organisms" (Bush, 2000, p. 469).

C_3 photosynthesizer- "The photosynthetic pathway used by most plants and all algae, in which the product of the initial reaction is phosphoglyceric acid, or PGA, a three-carbon acid" (Molles, 1999, p. 482).

Cation exchange capacity (CEC) – "readiness (often of a clay material) to swap cations for other ions (particularly hydrogen ions)" (Bush, 2000, p. 469).

Ecotypic differentiation- physiological differences, or plasticity, displayed by the same species at sites differing across some environmental variable (Anella & Whitlow, 2000).

Edaphic factors- pertaining to the soil.

Fitness- "The number of a parent's young that live to reproduce; divided by two if sexual reproduction is involved" (Bush, 2000, p. 471).

Grey-Brown Podzolic soil- one of two soil types dividing the North American deciduous forest, characterized as acidic with high organic matter and very fertile (Vankat, 1979).

Ion- "An atom or group of atoms that has lost or gained one or more electrons and, consequently, has acquired a positive or negative charge. Ions are designated by 1 or 2 superscripts following the chemical symbol" (Bush, 2000, p. 474).

Mycorrhizae- "The mycelia of certain fungi that grow symbiotically with the roots of some plants and provide for additional nutrient uptake" (Bush, 2000, p. 475).

Ozone- "A gas, O_3, that is a pollutant in the lower atmosphere but necessary to screen out ultraviolet radiation in the upper atmosphere" (Bush, 2000, p. 477).

pH- "scale used to designate the acidity or basicity (alkalinity) of solutions or soil. pH 7 is neutral; values decreasing from 7 indicate increasing acidity; values increasing from 7 indicate increasing basicity. Each unit from 7 indicates a 10-fold increase over the preceding unit" (Bush, 2000, p. 477).

Polar molecule- "The unsymmetrical distribution of electrons in a molecule that results when one atom attracts electrons more strongly than another" (McMurry, 2000, p. A-31).

Red-Yellow Podzolic soil- one of two soil types dividing North American deciduous forest, less fertile with reds in upland regions and yellows along the coast (Vankat, 1979).

Stomata- "Microscopic pores in leaves, mostly in the undersurface, that allow the passage of carbon dioxide and oxygen into and out of the leaf and that also permit the loss of water vapor from the leaf" (Bush, 2000, p. 480).

Succession- "the gradual, or sometimes rapid, change in species that occupy a given area, with some species invading and becoming more numerous while others decline in population and disappear. Succession is caused by a change in one or more abiotic or biotic factors benefiting some species but at the expense of others." (Bush, 2000, p. 480).

OVERVIEW OF CHAPTERS

Our ever-changing environment presents challenges to both the flora and fauna of local and global ecosystems. The east coast of the United States is home to a grouping of unique deciduous hardwood tree associations. A common member found throughout this range is *Acer rubrum* (red maple). A complex set of environmental variables involving chemical processes, geologic substrate, biological function, and ultimately governmental legislation are currently interacting to set the stage for potential ecosystem restructuring along the east coast. As a result of its hardiness, rapid growth rate, and range parameters, *Acer rubrum* may potentially become a more functional dominant throughout its current range and expand into areas dominated by other species, broadening its niche.

rapid growth rate, and range parameters, *Acer rubrum* may potentially become a more functional dominant throughout its current range and expand into areas dominated by other species, broadening its niche.

The intent of this study was to identify potential physiological parameters of *Acer rubrum* for acid precipitation. Once this parameter was assessed the current knowledge base will broaden and the likelihood of *Acer rubrum* as a potential successional-dominant, given future atmospheric and environmental degradation, may be extrapolated. The interrelationship between pH of precipitation and biomass allocation, both above and belowground, was explored through the review of literature and the laboratory research of this study. A detailed explanation of the methods and procedures employed in collecting the experimental data was then provided, followed by a comprehensive reporting of the research findings. Finally, conclusions as to the feasibility of the afore mentioned scenario was presented, as were suggestions for future research necessitated by questions arising from the current study.

CHAPTER II

REVIEW OF LITERATURE

Ecologists, botanists, chemists, and biologists have joined with mathematicians, statisticians, meteorologists, and politicians to amass an extensive array of research concerning both biotic and abiotic implications of acid rain on a large array of species and systems. Within the body of published literature are found distinct responses of *Acer rubrum* to varying environmental conditions across multiple treatment scenarios. Distinct survival advantages of *Acer rubrum* over competing species are unearthed, as well as basic findings related to altered pH and biotic physiology. Prior research findings lend strong credibility to the hypothesis that *Acer rubrum* holds real viability as a future competitive dominant. Lack of any existing research on altered pH and *Acer rubrum* above and belowground biomass allocation derives the need for the current research, as stated by Whittaker: "Comprehensive data is required to elucidate the effects of increased H^+ burden on the alteration of physio-biochemical mechanisms that cause a reduction in deciduous forest growth of the east" (Whittaker et al., 1974). In this literature review, the following topics will be touched upon: impacts of pH on biological systems, biotic responses of *Acer rubrum* to abiotic factors, and survival advantages of *Acer rubrum* on a community wide basis.

Impacts of pH on Biological Systems

Although some research purports that a survival advantage exists for coniferous species over deciduous, current trends in science find a distinct advantage in survival for deciduous species, with a pH range below 2.6 required to induce significant growth reductions (Percy, 1986). Many researchers have found conifers less sensitive to acid rain based on visible foliar effects rather than physiological functioning impairment (Evans, 1978; Cox, 1983). Concerning the specific morphology of *Acer rubrum*, no mean cotyledon length alteration, of significance, was noted at any pH range between 2.6-5.6, and leaf length and width was only significantly impacted at levels of 2.6 or below (Percy, 1986). For seedling germination the results are mixed; Percy found no impacts of reduced germination with lowered pH (Percy, 1986), while others have noted germination capacity inhibition with pH levels as high as 4.0 (Raynal et al., 1982). The generally accepted threshold of damage for biological systems is around pH 4.6, with the pH of many east coast sites frequently found in the 3.5-3.74 range (Percy, 1986).

Acid precipitation increases calcium mobilization by 226% and magnesium by 244%, moving these essential elements to deeper soil profiles (Lodhi, 1982). These nutrients would be less available to species unable to allocate compensatory belowground structures to overcome this circumstance. For example, research on herbaceous grass species noted a biomass decrease of 12.8 g/m^2 with a decline in pH from 6.6 to 4.0 (Lodhi, 1982). Though results vary widely depending on soil-parent material, type of litter, microbial populations, and tree species present, acid precipitation shows a positive relationship (as variable A decreases variable B decreases and vice-versa) with soil bacteria, nitrification, and overall plant biomass (Lodhi, 1982). Future systems may shift toward a semi-podzol (nutrient-poor) ecosystem. Deeper allocation of root biomass would prove advantageous for *Acer rubrum* in tapping deeper nutrient sources.

Many eastern species, currently in dominance, respond poorly to lowered pH precipitation. Oak species (*Quercus*) have been found to measure lowered xylem pressure potential, decreasing nutrient transport, at pH levels between 3.6-4.8 (Walker, 1993). This same species tends to have more acidic soils under their cover, while *Acer rubrum* maintains the least acidic soils (Shear & Stewart, 1934). This condition results from H^+ ions transported in via stomates being pumped out, while calcium is transported in for neutralization. Stomates of *Acer rubrum* transfer in less of the H^+ ions (Samuelson & Kelly, 1997). The primary damage to biological systems, due to lowered pH, is root damage resulting in water and nutrient uptake difficulties, even with yellowing of the foliage little chloroplast damage is noted (Ulrich et al., 1980).

Biotic Responses of *Acer rubrum* to Abiotic Factors

Increases in the pollutants responsible for acid rain are being compounded by increases in lower atmospheric ozone (O_3) (Bush, 2000). The literature contains several studies of interest, which point to competitive advantages maintained by *Acer rubrum* over other species. Research conducted in The Great Smoky Mountains National Park found that, when exposed to elevated ozone levels, the seedlings and mature trees of *Acer rubrum* (12 mmol m^{-2}) had lower stomatal ozone conductance than did cohort species, black cherry (26 mmol m^{-2}), and red oak (16 mmol m^{-2}) (Samuelson & Kelly 1997). This in turn resulted in greater decreases in photosynthesis and increased chloroplast damage in the cohort species relative to *Acer rubrum* (Samuelson & Kelly, 1997).

Global emissions of greenhouse gases are currently responsible for elevated atmospheric carbon dioxide (CO_2) levels (Bush, 2000). Plants take in carbon dioxide through their stomates, split water molecules, and recombine them with carbon dioxide to form sugars, with oxygen as a by-product. Increases in carbon dioxide lead to increases in biomass production in many species, which in turn leads to increased nitrogen and phosphorus uptake for biomass allocation (Bernston & Bazzaz, 1996). While some species change their allometry with increased carbon dioxide, *Acer rubrum* does not (Bernston & Bazzaz, 1996). Of 150 species studied, 87% were found to increase their root production while 41% altered their root mass relative to their shoot mass (Rogers et al., 1994). The average C_3 woody species was found to have a biomass enrichment ratio of 1.40 with the minimum ratio of 1.06 belonging to *Acer rubrum* (Poorter, 1993). These changes produce new plant growth patterns; local nutrient depletions; slower decomposition rates, with increases in the carbon to nitrogen ratios; and myriad other alterations in the plant community dynamics (Bernston & Bazzaz, 1996). Species with lower allometry changes would ultimately be favored over species with higher allometry alterations, in a succession scenario.

Many scientists forecast changes in the current precipitation amount patterns with changing global climate (Bush, 2000). Autotrophic species will need a certain degree of genotypic plasticity or highly variable tolerance levels to succeed. Research focusing on these types of changes has yielded interesting and highly relevant results in relation to *Acer rubrum*. In order to overcome flood stress, *Acer rubrum* was able to allocate nutrients to additional stem and leaf production, thereby increasing photosynthesis and sugar production (Day, 1987). Only conditions of continuous flooding produced reductions to root biomass; this was compensated for by increased production of adventitious roots that normalized nutrient intake (Day, 1987). Though not capable of phenotypic plasticity, like many lower autotrophs, *Acer rubrum* does exhibit ecotypic differentiation with a degree of genetic plasticity. Seedlings in dry sites fare better in drier conditions than do seedlings from wetter areas and vice-versa (Anella & Whitlow, 2000). Based on genetic variation among seedlings at those sites, these contrasting responses to variable edaphic factors account for Acer rubrums' wide range and impart a clear competitive advantage over less tolerant species (Anella & Whitlow, 2000).

Survival Advantages of *Acer rubrum* on a Community Wide Basis

Many factors account for the species composition found in a given ecosystem. Species that exhibit evolution of highly advantageous characters are likely to experience the greatest fitness. *Acer rubrum* has evolved reproductive traits with strong selection for early fruit development and rapid maturation in a large portion of its total population (Jones et al., 1997). Two to three week emergence differences may result in an order of magnitude difference in survival, persisting for several years (Jones et al., 1997). This imparts advantages for light capture in the understory, increasing biomass production of *Acer rubrum* relative to competing cohorts of later emerging species. *Acer rubrum* does emerge later in a small portion of the population;

this imparts survival insurance for relatively late occurring environmentally harsh events (Jones et al., 1997).

Many variables interact to influence species composition (seed dispersal pattern, resource declines, and herbivory); all produce community changes and may close the window open for succession of certain species (Gross, 1980). Within seven years of old-field abandonment, it was found that 90% of the community established *Acer rubrum* had invaded, while six other cohort species all had delayed invasion (Rankin & Pickett, 1989). The time of invasion greatly impacts the success of species in succession (Rankin & Pickett, 1989). The large seed crops and high shade tolerance of *Acer rubrum* seedlings give this species a decided advantage in colonization and succession (Hutkin & Yawney, 1961). The additional characteristic of wide variation of samara (seed) length, endosperm content, and seasonal mast crop among trees within the same area provides greater survivability to overcome disease, parasites, and predators specific to one type of prey (Townsend, 1972).

The vast majority of plant species form symbiotic relationships with soil fungi to facilitate more efficient nutrient exchange. The fungi, termed mycorrhizae, receive sugars from the host and afford greater nitrogen, phosphorus, and mineral uptake to the tree. The specific type and function of the mycorrhizal relationship has evolved over time to associate specific types of fungi with only certain tree species. *Acer rubrum* has been found to associate with endotrophic arbuscular mycorrhizae, which penetrates the root hair epidermis and grows horizontally through the cortical parenchyma (Medve, 1971). Changes in soil microbiology will favor species that form symbiotic relationships with endotrophic arbuscular mycorrhizae rather than ectomycorrhizal species (O'Neill & Norby, 1988). Species such as *Liriodendron tulipefera* and *Acer rubrum* associate with mycorrhizae capable of compartmentalizing aluminum and other caustic elements, preventing them from entering the trees' tissues (O'Neill & Norby, 1988). These relationships provide *Acer rubrum* a clear competitive advantage as acid rain changes the cation exchange ratio of local soils.

Summary

Changes is global climate will favor hearty adaptable species capable of withstanding a wide variety of environmental conditions. *Acer rubrum* has been extensively researched across many of these potential environmental variables. Under conditions of elevated O_3, CO_2, and precipitation, as well as decreased precipitation *Acer rubrum* has shown competitive advantages over cohort species. Though certain aspects of pH and *Acer rubrum* have been addressed, research on biomass allocation both above and belowground is lacking. Understanding any allometry changes under conditions of lowered pH precipitation is valuable in predicting what species will come to dominate future systems. The current study will address a key facet of this question. Chapter III will present the detailed methods and procedures used in this experiment.

CHAPTER III

METHODS AND PROCEDURES

This was an experimental study to determine the responses of *Acer rubrum* seedlings to differing levels of pH precipitation. The current chapter yields a detailed analysis of the methods and procedures implemented to collect this data. The sub-sections of this chapter include: population, research variables, laboratory procedures, methods of data collection, statistical analysis, and summary.

Population

The experimental population for this study consisted of 100 *Acer rubrum* (red maple) seedlings obtained from the Commonwealth of Virginia Department of Forestry, Augusta Forestry Center, grown is Crimora, Virginia. The growing conditions for these seedlings, prior to shipping, were consistent and representative of much of the current range for *Acer rubrum*. The red maple, also referred to as the "scarlet maple" or "swamp maple", is a large tree with a narrow or rounded, compact crown reaching 90' with an average diameter of 2.5', at this height (Little, 2001). An attractive shade tree, it has the greatest north-south distribution of all East Coast tree species (Little, 2001).

Research Variables

The three variable treatments implemented in this study consisted of reduced pH water administered at: (1) pH 2.61, (2) pH 3.44, (3) pH 4.48, and (4) a control group approximating "neutral" rainwater of pH 6.53.

Lab Procedures

Upon receipt 80 seedlings were randomly selected and potted in 6.5 in. diameter plastic pots. The substrate consisted of 25 mm/pot Hoffman Horticultural Perlite and 125 mm/pot Miracle Grow Potting Mix with 60% Canadian Sphagnum Peatmoss. The soil nutrient composition, at potting, was .21% total nitrogen (.12% ammoniacal nitrogen, .09% nitrate nitrogen), .07% available phosphate (P_2O_5) and .14% soluble potash (K_2O). The seedlings were normalized by height and distributed into four groups (n=20) across the greenhouse floor. The seedlings were afforded a greenhouse acclimation period of 24 days. During this period 200 mL of de-ionized water (1 drop/5 gallons .10% NaOH added for pH stabilization, average pH 6.22) was administered, twice a week to each seedling.

Following the acclimation period, appropriate pH levels were obtained for the treatment groups by addition of HCL (pH 2.61 – 6.75mL HCL/gallon H_2O, pH 3.44 – 1mL HCL/gallon, pH 4.48 – 1 drop HCL/gallon, and pH 6.53 – 1 drop NaOH/5 gallons). The pH was monitored using pH Testr 3 ™ with ATC. Prior to adjustment, all water used in the experiment was de-ionized. All samples received appropriate treatments twice per week for 12 weeks. All other potential parameters were held constant (light availability, temperature, substrate, etc.).

Methods of Data Collection

A baseline biomass for both above and belowground was initially obtained by randomly selecting 20 seedling samples. The above and belowground portions for each seedlings' allometric distribution was separated, placed in individual sterile paper bags, dried for 48 hours at 70°C, to a constant weight, using a Model 603 NAPCO Desiccating Oven, and weighed using a Model XD-800 Denver Instrument Company Scale, to the nearest hundredth of a gram. An additional ten bags were dried to allow an average bag weight to be obtained for future data analysis.

Treatment and control samples were mechanically harvested on June 6, 2005. The above and belowground portions were separated following belowground biomass separation from substrate, utilizing deep sink washing through a 1mm mesh screen. The samples were then placed in sterile paper bags, dried to a constant weight and weighed.

Statistical Analysis

Standard statistical analysis using a t-test was used to determine the significance, $p>(.01)$, of each treatment variable relative to the control pH 6.53 group. In addition to analysis of post treatment biomass allocation across all samples, total biomass allocation relative to initial biomass was also analyzed using the t-test.

Summary

The current chapter described the exact methods and procedures used in obtaining the data required to address the stated problem. Chapter IV presents precise data found during the experiment, while Chapter V summarizes the study, reaches conclusions concerning this research, and potential areas of future research.

CHAPTER IV

FINDINGS

The problem of this study was to determine the short-term impacts of lowered pH precipitation on above and belowground biomass allocation in *Acer rubrum* seedlings. This chapter examines the data procured from the treatment trials. The findings address above, below, and cumulative biomass allocations across all administered pH ranges. Additionally, data on the substrate buffering capacity, following experimentation, and the allometric variations in biomass distribution are presented.

Above, Below, and Cumulative Biomass Allocation

Following the initial greenhouse acclimation period of 24 days all treatments were commenced. Within 6 days of initial treatment administration all samples had begun to break their apical buds and leaf out. The average temperature range for the month of March, during dormancy break, was 13.2 – 42.5^0 C. The range for April was 14.8 – 48.1^0 C, while the range for May and the first week of June was 15.3 – 50.12^0 C.

The average gram weight aboveground biomass for all treatment groups had a positive correlation with the pH of the sample. Aboveground components were obtained upward at the point where the shoot produced its first root. The range for the aboveground components was 3.526 grams – 12.632 grams for the control group. The aboveground samples included the leaf biomass weights. (See Figure 1.)

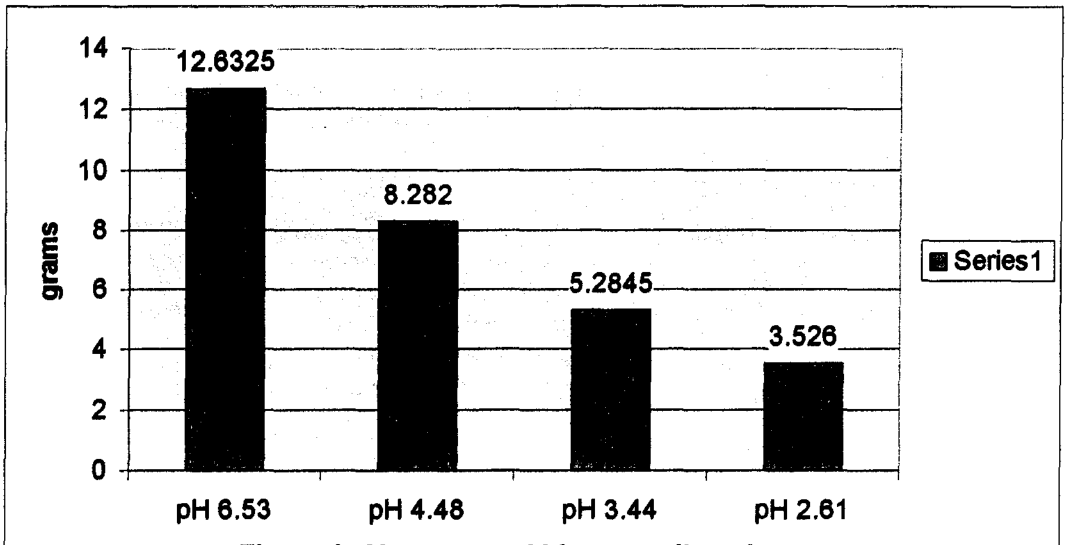

Figure 1. Aboveground biomass allocation

The distribution of belowground structures across all treatment groups showed a positive correlation with the pH of the group. The baseline data for the belowground biomass (not pictured) produced an initial average of 1.009 grams, prior to acclimation and treatment. As with the aboveground data, the measures shown represent sample data minus the average bag weight of 6.904 grams. Again, as with the aboveground components the control group shows a notable disparity in allocation relative to the lowered pH groups. The belowground allocations ranged from a low of 1.98 grams at pH 2.61 to a high of 4.77 grams at pH 6.51. (See Figure 2).

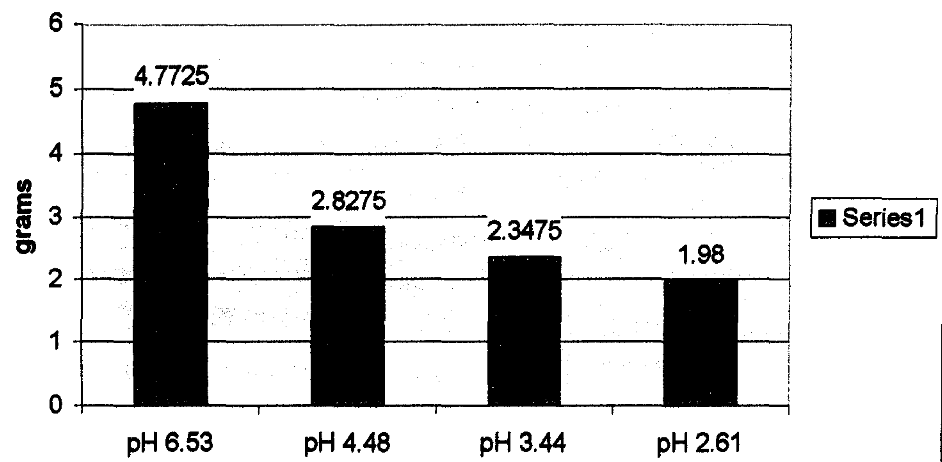

Figure 2. Belowground biomass allocation

The relative overall biomass allocation comparison, inclusive of both the above and belowground structures revealed a positive correlation between the pH administered to the treatment group and the overall biomass allocation. The total biomass range was 17.405g for the 6.53 pH to 5.508g at the 2.61 pH level. (See Figure 3).

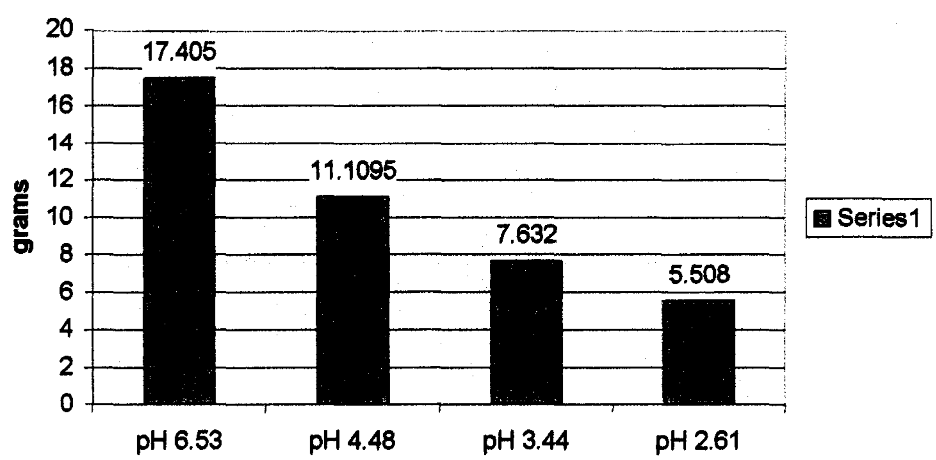

Figure 3. Total biomass allocations

Substrate Buffering and Allometric Variations

Upon completion of all trials, a substrate buffering capacity test was administered. With an initial pH of 4.02, sample water was drained through each of the sample substrates to assist in accounting for edaphic factors related to biomass results. The neutralization ability of the sample soils was .30 for the pH 2.62 group, .61 for the pH 3.44 group, .94 for the pH 4.48 group, and 1.09 for the pH 6.53 group. (See Figure 4).

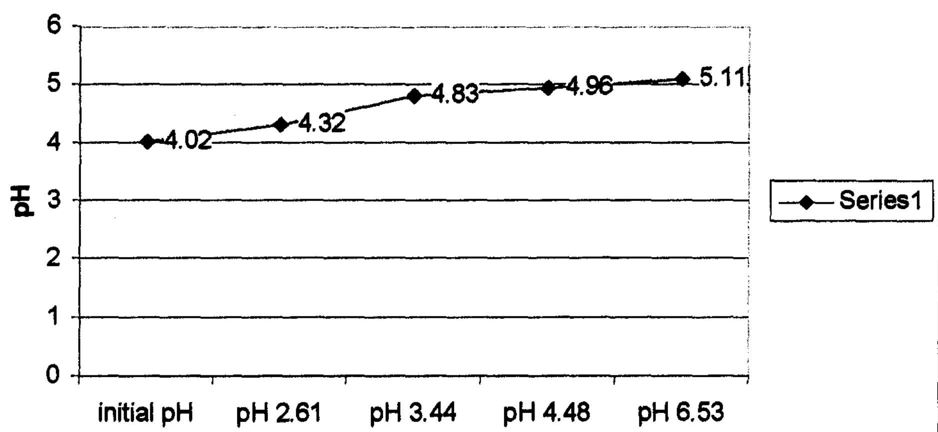

Figure 4. Buffering capacities of substrate following trials

The percentage of allocation for both above and belowground structures for each treatment group showed an approximate 75% allocation for aboveground structures relative to 25% allocated to belowground structures. While the pH 6.51 and 4.48 groups showed an average belowground allocation of 26.43%, the pH 3.44 and 2.61 groups averaged 33.34% to these same structures. Visual inspection of all samples revealed necrosis and mottled yellowing of foliage for the pH 2.61 group across approximately 60% of that sample with death occurring in fully 15% of the sample seedlings, at pH 2.61. (See Figure 5).

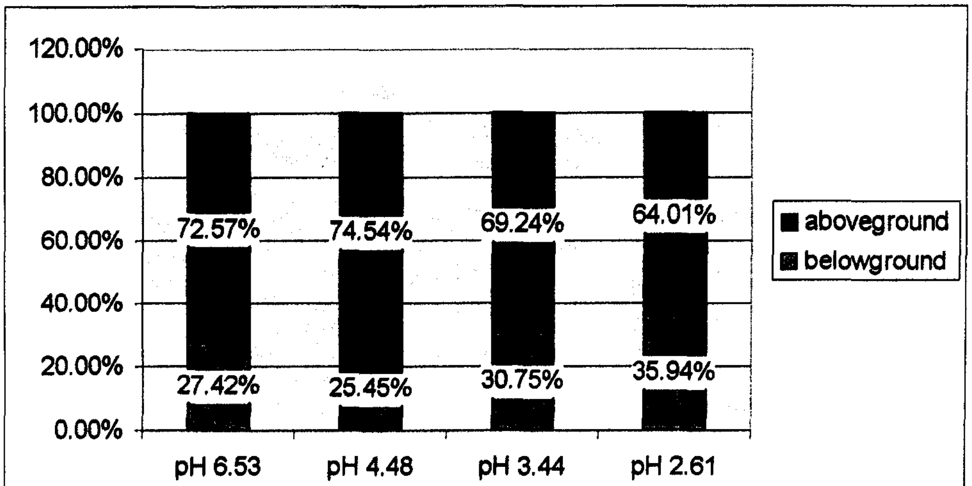

Figure 5. Allometric distributions

Table 1 presents the means and t-test scores for both above and belowground biomass allocation across all sample groups.

Table 1. Means and t-test values

	Group mean (grams)	t-test
Aboveground		
pH 6.51	12.63	
pH 4.48	8.28	2.307
pH 3.44	5.28	4.27
pH 2.61	3.52	5.498
Belowground		
pH 6.51	4.77	
pH 4.48	2.82	2.346
pH 3.44	2.34	3.34
pH 2.61	1.98	3.884

These scores are in relation to the pH 6.51 control group. The critical value of 2.4298 at $p>(.01)$, for a one-tailed test was attained for the pH 2.61 and 3.44 groups, both above and belowground. At the pH 4.48 this value was not attained. However, at $p>(.05)$ the pH 4.48 group does reach the critical value of 1.686.

Summary

The research goal of the present study was to determine the impact of pH on the above and belowground biomass distributions in *Acer rubrum* seedlings. Data collected support a significant response across these parameters when applying the one-tailed critical values of t (2.4298) for pH 2.61 and 3.44 groups. When the $p>(.05)$ level is considered all groups show a statistically significant response to pH treatments. Chapter V provides a comprehensive summary of the current study with conclusions about the data delivered in Chapter IV and recommendations derived from the data pertaining to both the current research and potential future endeavors.

CHAPTER V

SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

The present chapter will present an overview of this study. Additionally, the research data will be evaluated to determine the extent to which the initial research questions were addressed via the collected data. Once the validity of the research hypothesis has been addressed, attention will focus on suggestions concerning future research about the potential of *Acer rubrum* in forest succession.

Summary

The current research initially set forth the problem of determining the short-term impacts of lowered pH precipitation on above and belowground biomass allocation in *Acer rubrum* seedlings. It was hypothesized that a positive correlation exists between the lowering of precipitation pH and the biomass allocation in *Acer rubrum*. Current environmental changes, both anthropogenic and natural, necessitate a better comprehension of potential directions for future conservation programs. Understanding the responses of *Acer rubrum* to lowered pH precipitation affords a working knowledge of its potential as a future successional dominant in the east. While the sample population provides a glimpse of potentialities for this species, it by no means is inclusive of all genetic constituents.

A complete analysis drawing upon the current body of literature available on previously conducted research on the red maple has been presented. Through thorough examination of previously published literature, a comprehensive analysis of the impacts of pH on biological systems and the particular responses of *Acer rubrum* to these, as well as other mitigating abiotic factors, is conducted. The structure of the community ecosystem where *Acer rubrum* currently finds its range was considered with respect to the characteristics of *Acer rubrum* that currently account for that success. Ultimatley, the lack of any comprehensive data in the literature on pH alteration and *Acer rubrum* was exposed, addressing the need for the current research.

The origin of the 100 *Acer rubrum* seedlings used for the current research was Crimora,Virginia. The pH administrations at 2.61, 3.44, 4.48, and 6.53 were addressed as were the complete methods and procedures implemented in the research study. The experimental group, experimental procedures, and the methods of data collection were also detailed.

The data collected via the research was thoroughly presented. All findings on the above and belowground biomass allocation following treatment were presented. Additionally, the data relevant to the averages and t-tests have put forth. A final overall allometric distribution was presented along with the final buffering capacities of the study substrates.

Conclusions

The response of *Acer rubrum* to lowered pH showed a highly significant variation, both for below and aboveground biomass allocation. The data collected present a strong case for the acceptance of the initial predictive hypothesis that:

H_1: From a neutral pH of 7, there is a positive correlation between the lowered pH of precipitation and the above and belowground biomass allocation in *Acer rubrum* seedlings.

The findings of this study indicate that *Acer rubrum* does show significant promise as a successional dominant in edaphically challenged soils at or above pH 4.48. When conditions reach pH levels at or below 3.44 significant resources are allocated to elimination of H^+ ions. Soils unable to provide the appropriate buffers will correlate to significant reductions in potential biomass allocation for *Acer rubrum*. Were pH levels to maintain over significant time intervals at or below 2.61, it is likely that *Acer rubrum* would incur high mortality rates.

It does appear that below the acid heartiness level of pH 4.48 ($t=2.307$) *Acer rubrum* seedlings potentially alter their allometry in an attempt to more readily obtain nutrients critical to buffering (27.42% versus 35.94%). A noteworthy caveat to this finding is the fact that overall belowground allocation was significantly lower for the pH 2.61 group relative to the pH 6.51 group ($t=3.884$). Hence, this disparity may directly result from an inability to allocate any resources to aboveground structures rather than any change in the distribution of available resources.

Recommendations

Understanding which species are likely to constitute our future forests is of the utmost importance in planning and conservation. The timber and recreational value of these species may only then be fully achieved. The natural mycorrhizal associations found with many *Acer rubrum* communities afford the tree the ability to avoid harmful aluminum uptake. These very associations may impart the necessary competitive advantage required by this species at pH levels below 4.48.

While the current research provides credible data for the acid heartiness of *Acer rubrum* at certain levels, it does not address concerns about these conditions in natural settings. Future research delving into pH alterations in soils with *Acer rubrum* seedlings inoculated with endoarbuscular mycorrhizae would vastly improve our understanding of how natural systems might potentially react. Additionally, research comparing allometric distributions over long-term pH alterations in soils of varying buffering capacities would greatly assist in the comprehension of how allocation to below and aboveground structures is related to differing edaphic factors in *Acer rubrum*.

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